



## ***Pelagia benovici* sp. nov. (Cnidaria, Scyphozoa): a new jellyfish in the Mediterranean Sea**

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### **Abstract**

A bloom of an unknown scyphistome jellyfish species was recorded in the North Adriatic Sea from September 2013 to early 2014. Morphological analysis of several specimens showed distinct differences from other known scyphistome species in the Mediterranean Sea and unquestionably identified them as belonging to a new pelagiid species within genus *Pelagia*. The new species is morphologically distinct from *P. noctiluca*, currently the only recognized valid species in the genus, and from other doubtful *Pelagia* species recorded from other areas of the world. Molecular analyses of mitochondrial cytochrome *c* oxidase subunit I (COI) and nuclear 28S ribosomal DNA genes corroborate its specific distinction from *P. noctiluca* and other pelagiid taxa, supporting the monophyly of Pelagiidae. Thus, we describe *Pelagia benovici* sp. nov. Piraino, Aglieri, Scorrano & Boero.

**Key words:** taxonomy, new species, scyphomedusae, invasive alien species, North Adriatic, jellyfish blooms

### **Introduction**

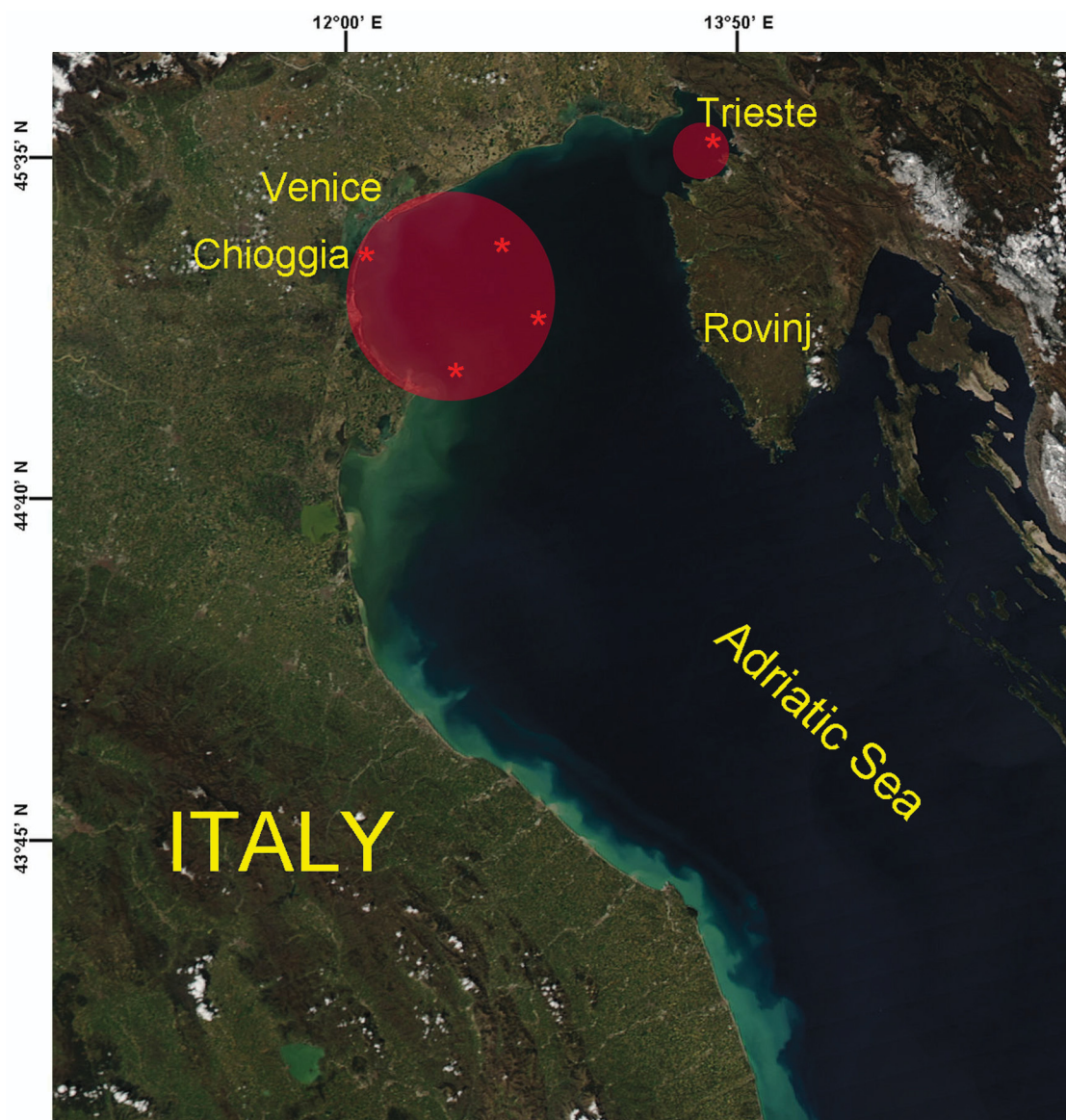
The impact of jellyfish blooms on fisheries and other human activities renewed scientific attention to the most conspicuous taxon of marine gelatinous organisms, the Scyphozoa (Boero 2013). In the Mediterranean Sea, the mauve stinger *Pelagia noctiluca* (Forskål 1775) occurred in mass throughout the western and central basins including the Adriatic Sea since the early 1980s, becoming the most important jellyfish because of its widespread distribution, high abundance, and ecological role (CIESM 2001; Brotz 2012; Canepa *et al.* 2014). However, the overall biodiversity of Scyphozoa remains poorly understood, with several unknown life cycles, multiple taxonomic synonymies, and assumptions of cosmopolitan species that either under-estimate or over-estimate diversity. Species new to science (Galil *et al.* 2010) and the description of new anatomical internal structures in well known taxa, such as the quadralinga in the Pelagiidae (Gershwin & Collins 2002), are common, suggesting much remains to be learned.

Here we report on a so far undescribed jellyfish species in the Mediterranean Sea, off the North Adriatic coasts, in the gulf of Venice. A prolonged bloom with densities of hundreds of mature medusae per trawl (2.5 nautical miles, 35 minutes, 35 x 5 m<sup>2</sup> net mouth) has been observed by fishermen and divers from mid September 2013 to at least March 2014; the bloom occurs near the Po River Delta to the Gulf of Trieste, at depths from the surface to 20–25 m. Morphologically the jellyfish can be referred to the scyphozoan order Semaestomeae, and the family Pelagiidae, genus *Pelagia*. However, the specimens differ morphologically from *P. noctiluca*, which currently is considered the only valid species in the genus (Cornelius 2013; Gul & Morandini 2013). Because there is a long history of species introductions to the Mediterranean, we also make comparisons with *P. flaveola* Eschscholtz 1829 and *P. panopyra* Péron & Lesueur 1809, two *nomen dubium* from the Indian and Pacific oceans, and consider DNA

sequence variation that may distinguish the new species from *P. noctiluca* and other Semaestomeae. We conclude that a new *Pelagia* species, *Pelagia benovici* **sp. nov.** Piraino, Aglieri, Scorrano and Boero, now occurs in the Mediterranean Sea.

## Material and methods

Blooms of the new jellyfish species were observed from mid-September 2013 to at least March 2014 in the Gulf of Venice, mainly in the fishery grounds near Chioggia (approximately 45° 20' – 45° 5' N) from the coastline to several miles offshore (13° 02'E), as well as within the Venice Lagoon (Fig. 1). Hundreds of specimens were collected as by-catch during diurnal fish trawls at 20–25 m depth. Five living specimens also were observed and photographed underwater on December 5th near the sea surface at 6:00 a.m. near Muggia (Gulf of Trieste; 45° 36' N, 13° 43' E). Additional records were made on 20<sup>th</sup> January, 2014 in shallow waters near Chioggia. Twenty specimens were preserved in 4% formaldehyde solution in seawater for morphological analyses or in 95% ethanol for DNA analysis.



**FIGURE 1.** Map of sampling sites (stars) and observed distributional range (circles) of *Pelagia benovici* **sp. nov.** in the North Adriatic Sea.

Morphological analyses were based on relevant characters as defined by Russell (1964, 1970), Mianzan & Cornelius (1999), Gerswhin & Collins (2002), and Morandini & Marques (2010): shape and coloration of umbrella; shape and distribution of cnidocyst warts; thickness of mesoglea; shape and number of marginal lappets; morphology and number of rhopalia; number, arrangement and relative length of tentacles; presence or absence of muscular folds in tentacle sections; relative length and shape of manubrium and oral arms; shape and arrangement of radial septa in the gastrovascular cavity; number and shape of gonads; cnidocyst types and sizes. The cnidome was investigated on specimens preserved in formaldehyde solution by squash preparation of pieces of tentacles, umbrellar warts, and gonads under cover slips at 1000x using a Zeiss Axioskop microscope. Terminology of the cnidome followed Weill (1934), Mariscal (1974), Östman & Hydman (1997), and Östman (2000).

**DNA extraction, amplification and sequencing.** Total genomic DNA was extracted from ethanol preserved tissues, following a CTAB-phenol-chloroform based protocol (Dawson *et al.* 1998; Dawson & Jacobs 2001). Polymerase chain reactions (PCR) were performed in an Eppendorf Mastercycler Gradient thermal cycler. Mitochondrial cytochrome *c* oxidase subunit I (COI) was amplified using the primers LCOjf (Dawson 2005) and HCO2198 (Folmer *et al.* 1994) using the following profile: 94°C for 4 min, 51°C for 2 min, 72°C for 2 min, 94°C for 4 min, 51°C for 2 min, 72°C for 2 min; 33 cycles of 94°C for 45 sec, 50°C for 45 sec, 72°C for 60 sec; final extension at 72°C for 5 min and refrigeration at 4°C. 28S rDNA was amplified with the primers Aa\_L28S\_21 and Aa\_H28S\_1078 (Bayha *et al.* 2010) using the reaction conditions suggested by the authors.

The size and quality of PCR products were examined on 1.5% agarose gels stained with GelRed™ and then purified with DE-001 GEL/PCR extraction and purification kit (Fisher Molecular Biology). The purified products were used as template DNA for cycle sequencing reactions performed by Macrogen (Korea). Both DNA strands were sequenced.

**Data analyses.** A total of seven COI and nine 28S sequences were obtained from *P. benovici* and compared with six (COI) and four (28S) sequences belonging to *P. noctiluca* from the Mediterranean Sea. Additional COI sequences of *P. noctiluca* from the Mediterranean Sea (Stopar *et al.* 2010) and Southern Atlantic Ocean (Miller *et al.* 2012) were included in the analyses, as well as COI sequences of *Pelagia* cf. *panopyra* (*nomen dubium*) from West Papua (Indonesia) and sequences from most Semaestomeae families downloaded from GenBank and used as references (Table 1); all new sequences were deposited in GenBank (Table 1).

Sequences were viewed and edited with 4Peaks (<http://nucleobytes.com/index.php/4peaks>) and contigs were assembled using Cap3 (Huang & Madan 1999). Sequences were verified using the “Barcode of Life Data Systems (BOLD)” Identification System (IDS) and matching them against the Nucleotide collection (nr/nt) database of NCBI, using the BLASTN search algorithm. Alignments were made with CLUSTALX version 2.0 (Thompson *et al.* 1997) using 10 as gap opening and 0.1/0.2 as pairwise and multiple extension penalties. The alignments were edited using MacClade 4.08a (Maddison & Maddison 2005). MEGA 5.2 (Tamura *et al.* 2011) was used to calculate genetic distances among sequences using the Kimura 2-parameter (K2P; Kimura 1980) model. Neighbor-joining (NJ) and Maximum Likelihood (ML) trees were constructed based on the K2P model in MEGA 5.2. Bootstrap values were calculated using 1,000 iterations. Nucleotide substitution models implemented in the phylogenetic analyses were determined using jModelTest 2.1.4 (Guindon and Gascuel 2003, Darriba *et al.* 2012), based on the Akaike Information Criterion (AIC). The preferred models were the General Time Reversible model with gamma distributed rate variation among sites (GTR+G) for COI and GTR+G+I for 28S. Bayesian Markov chain Monte Carlo (MCMC) analyses were performed with MrBayes 3.2.2 (Ronquist and Huelsenbeck 2003) setting the *Aurelia* sp. sequence as outgroup both for COI and 28S. The analyses were run for two million generations, using five hundred thousand generations as burn-in and sampling every one hundred generations. In the Bayesian analyses for COI all third codon positions were excluded because evidence of sequence saturation was observed.

**Nomenclatural acts.** This published work and the nomenclatural acts it contains have been registered in ZooBank, the online registration system for the ICZN. The ZooBank LSIDs (Life Science Identifiers) can be resolved and the associated information viewed through any standard web browser by appending the LSID to the prefix “<http://zoobank.org/>”. The LSID for this publication is: urn:lsid:zoobank.org:pub:39B361C0-FCFD-4398-883E-6EC576B13EC8.



## Results

### Order Semaestomeae

#### Family Pelagiidae Gegenbaur 1856

##### Diagnosis of the genus *Pelagia* Péron & Lesueur 1809 (redefined, after Gershwin & Collins 2002)

Pelagiidae with exumbrella covered by conspicuous cnidocyst warts; eight marginal tentacles alternating with eight marginal sense-organs located on shallow sensory pits; 16 marginal lappets; 16 unbranched, simple radial septa terminating between sense organs and tentacles, dividing the gastrovascular sinus into 8 tentacular and 8 rhopalial separate pouches that do not communicate with neighbouring pouches at the umbrella margin. Direct life cycle without polyp stage.

Type species: *Pelagia noctiluca* (Forskål 1775)

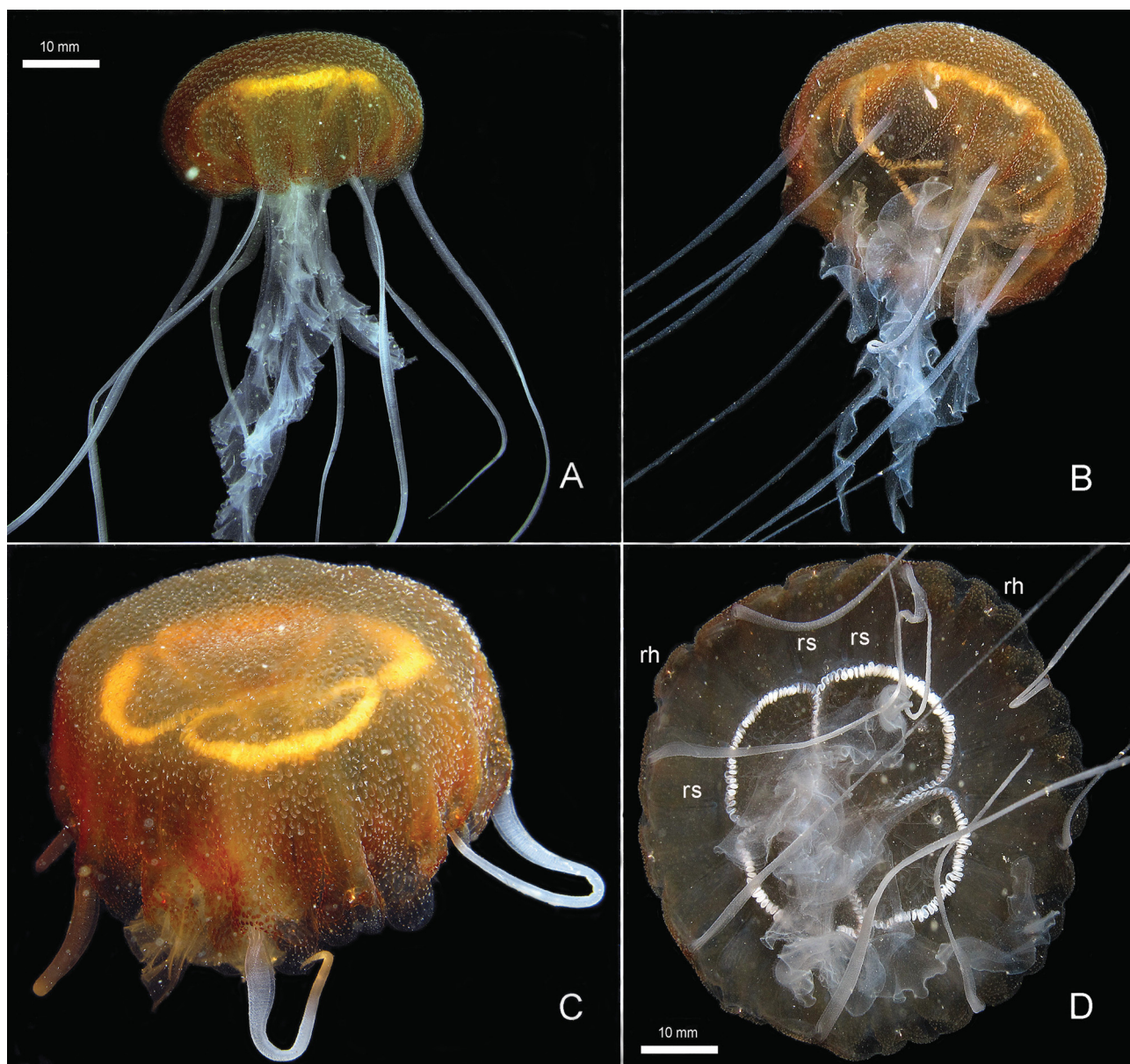
##### *Pelagia benovici* Piraino, Aglieri, Scorrano & Boero sp. nov.

Holotype: male specimen (adult), collected from Gulf of Venice (Chioggia), November 2013, 46 mm bell diameter. Deposited in the Collection of the Museum of Adriatic Zoology Giuseppe Olivi (Palazzo Grassi, Chioggia, University of Padova). Accession number: CN54CH.

Paratype I: female specimen (adult), Gulf of Venice (Chioggia), November 2013, 50 mm bell diameter. Deposited in the Collection of the Museum of Adriatic Zoology Giuseppe Olivi (Palazzo Grassi, Chioggia, University of Padova). Accession number: CN55CH. Other material: 10 specimens. Gulf of Venice (Chioggia), November 2013 32–45 mm (range of bell diameter). Deposited in the Collection of Marine Invertebrates at the Laboratory of Zoology and Marine Biology of the University of Salento (Lecce). Accession numbers: UNIS\_SCY\_001–10. Five specimens photographed in the field, but not collected, by Mr. Fabrizio Marcuzzo at Punta Sottile, Muggia (Trieste) on December 5<sup>th</sup>, 2013

**Description** (based on holotype and paratype). Preserved medusa almost flat, with thin transparent mesogleal jelly. Live specimens hemispherical during active swimming strokes (Fig. 2A). Exumbrella yellow-ochre in colour, homogeneously covered by prominent cnidocyst warts of various shapes, from rounded to oval to pointed, with whitish refringent tip of wart. Sixteen marginal lappets, rectangular, with rounded corners (Figs. 2B–D, 3A). Eight adradial, hollow, white and transparent tentacles, up to three times the diameter of umbrella in length. Large tentacle bases laterally compressed to ovoid, with medio-peripheral main axis, distally tapering into cylindrical shape (Figs. 2A–D, 3A). Absence of longitudinal muscular foldings in tentacle mesoglea (Fig. 3B). Eight marginal sensory organs (Fig. 2D), lacking ocelli, each located in a shallow pit formed by ectodermal outgrowth of the umbrellar margin and by overlapping sides of marginal lappets (Fig. 4A). Well-developed coronal muscle on subumbrellar surface. Simple radial septa terminating between sense organs and tentacles, dividing the gastrovascular sinus into 8 tentacular and 8 rhopalial separate pouches, tentacular pouches slightly larger than rhopalial ones (Fig. 4B). Stomach without gastric septa, with bundles of gastric filaments arranged in interradial groups, originating at the transition between stomach and gastrovascular sinus (Fig. 4C). Four interradial, elongated milky white, ribbon-like gonochoric gonads (holotype: male; paratype: female), horse-shoe shaped, convex; ribbon protrudes out of subumbrellar surface at periphery of gastric pouches; each ribbon spans two tentacular and one intermediate rhopalial pouch (Figs. 2D, 3A, 4B–D). Manubrium whitish, transparent,  $\leq 1.5$  times the diameter of umbrella in length, with very short oral tube and long delicate oral arms with frilled edges (Figs. 2A,B,D), covered by colourless cnidocyst warts (Fig. 4C). Perradial subumbrellar surfaces between gonads covered by brownish cnidocyst warts, smaller than exumbrellar warts (Fig. 4B, C), also scattered over the gonad foldings (Fig. 4D, 5A,B).

Cnidome (Fig. 5C–F). At least 3 cnidocyst types: holotrichous O-isorhizas (spherical, length 7–11  $\mu\text{m}$ ; width 7–11  $\mu\text{m}$ ), microbasic euryteles (ovoid, length 9–11  $\mu\text{m}$ ; width 4–5  $\mu\text{m}$ ), and a third larger type, provisionally identified by light microscopy as heterotrichous microbasic birhopaloid II type (ovoid, length 16–24  $\mu\text{m}$ ; width 14–17  $\mu\text{m}$ ).



**FIGURE 2.** *Pelagia benovici* sp. nov. (A) Lateral view. (B) Sublateral view. (C) Aboral view, with prominent exumbrellar cnidocyst warts. (D) Oral view, showing the distinctive horse-shoe shaped white gonads, and overall subumbrellar morphology. rh: rhopalia, rs: radial septa (for clarity, only few labels added).

**Etymology.** The species is named after the late Prof. Adam Benovic, who dedicated his life to the study of gelatinous plankton, especially in the Adriatic Sea.

**Type locality.** Gulf of Venice, Adriatic Sea, Mediterranean Sea.

**Molecular analysis.** Seven COI sequences from *P. benovici* specimens were identical to each other (mean within-species pairwise K2P distance = 0.001, S.E. = 0.001), and significantly dissimilar from the 14 *P. noctiluca* sequences from Mediterranean Sea and South Atlantic Ocean specimens (mean between-species pairwise K2P distance = 0.362, S.E. = 0.040) and from 8 *P. cf. panopyra* sequences (mean between-species pairwise K2P distance = 0.342, S.E. = 0.038). (Table 1). Unrooted NJ and ML trees had the same topology (data not shown) including consistently distinct *P. noctiluca* and *P. benovici* clades. The COI Bayesian tree (Fig. 6A) also shows consistent separation of *P. benovici* sp. nov. from any of its morphologically closest relatives, *P. noctiluca* and *P. cf. panopyra*.

Bayesian phylogenetic analysis of 28S (Fig. 6B) consistently showed the same topology as the NJ and ML analyses of 28S (not shown), including monophyly of the Pelagiidae, with all representatives of *Pelagia*, *Chrysaora* and *Sanderia* that were considered. However, this tree does not show reciprocal monophyly of *Pelagia*

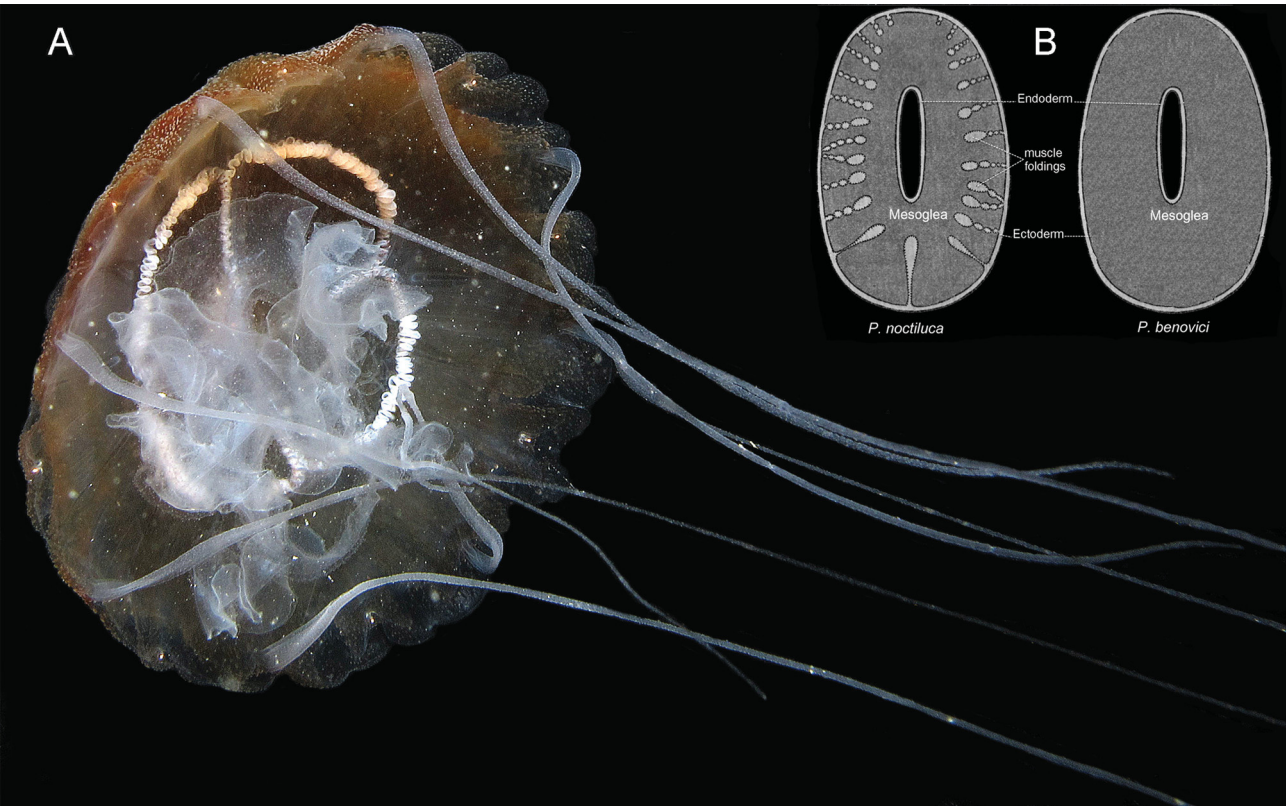


*benovici* **sp. nov.** and *P. noctiluca*; 28S sequences from medusae that were distinguished morphologically as different species occur together in two distinct clades.

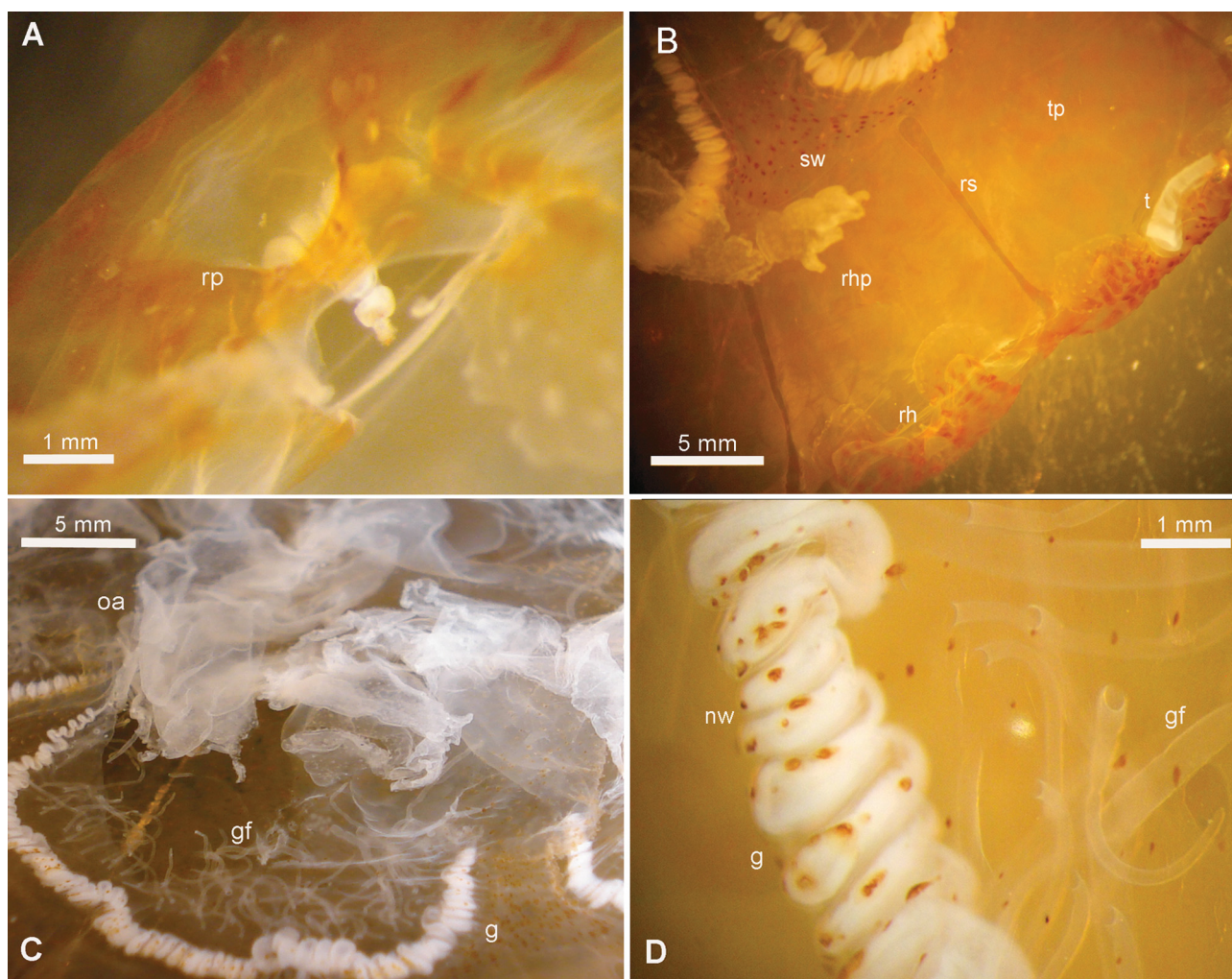
**TABLE 1.** List of taxa and number of individuals used for molecular analyses of COI and 28S markers and accession numbers of sequences.

Taxon	COI n	GenBank accession no.	28S n	GenBank accession no.
<i>Pelagia benovici</i>	7	KJ573409-KJ573415	9	KJ573396-KJ573404
<i>Pelagia noctiluca</i>	6	KJ573416-KJ573421	4	KJ573405-KJ573408
<i>Pelagia</i> cf. <i>panopyra</i>	8	KJ573422-KJ573429	n/a	n/a
<i>Pelagia noctiluca</i> *	8	GQ375927-30 JQ697960-63	1	HM194865
<i>Aurelia</i> sp.*	1	AY903187	1	EU276014
<i>Phacellophora camtschatica</i> *	1	GQ120099	1	AY920778
<i>Chrysaora</i> sp.*	1	DQ083524	n/a	n/a
<i>Chrysaora melanaster</i> *	1	FJ602545	1	HM194864
<i>Chrysaora fuscescens</i> *	n/a	n/a	1	HM194868
<i>Rhizostoma pulmo</i> *	1	HQ902121	1	HM194848
<i>Cyanea capillata</i> *	1	JX995344	1	HM194873
<i>Cyanea annaskala</i> *	1	AY902915	1	HM194831
<i>Cyanea lamareckii</i> *	1	HF930523	n/a	n/a
<i>Desmonema</i> sp.*	n/a	n/a	1	HM194857
<i>Sanderia malayensis</i> *	n/a	n/a	1	HM194861

\* Sequences downloaded from GenBank; n/a: not available.



**FIGURE 3.** *Pelagia benovici* **sp. nov.** (A) Oral view with extended marginal tentacles. (B) Sections of tentacles bases showing occurrence of longitudinal muscular foldings in the tentacles of *P. noctiluca* (left) compared with absence from *P. benovici* tentacles (right). Drawings after Krasinska, 1914 (modified).

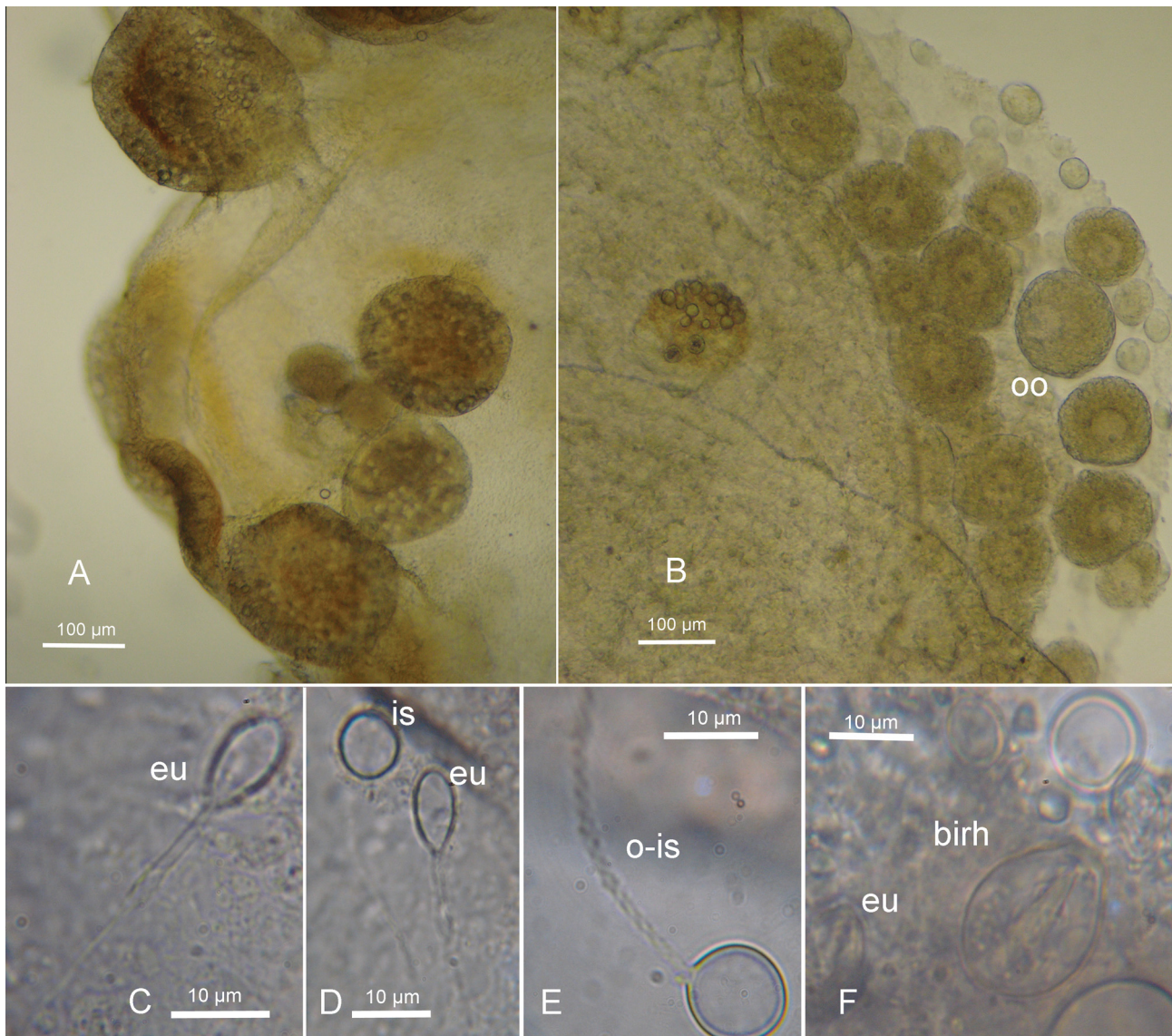


**FIGURE 4.** *Pelagia benovici* sp. nov. (A) Rhopaliar pit (rp). (B) Portion of gastrovascular sinus showing radial septa (rs), subumbrellar perradial warts (sw), tentacular (tp) and rhopaliar (rhp) pouches, rhopalia (rh), and rounded marginal lappets. (C) Gastric filaments (gf), horse-shoe shaped gonads (g), and oral arm (oa) covered by transparent warts. (D) Enlargement of ribbon-like gonad (g) covered by cnidocyst warts (nw) bordering the stomach wall with emergent gastric filaments (gf).

**Discussion and systematic remarks.** Within the order Semaestomeae, four families are widely recognized (see Kramp 1961; Russell 1970; Bayha & Dawson 2010)—Pelagiidae, Cyaneidae, Ulmariidae and Drymonematidae—and a fifth family, Phacellophoridae, has been proposed by Straehler-Pohl *et al.* (2011). The Pelagiidae are easily recognized by emergence of the tentacles at the umbrella margin, the absence of branched pouches of the gastrovascular sinus, and the absence of a ring canal.

Pelagiidae contains three genera (Gershwin & Collins 2002)—*Chrysaora*, *Pelagia*, and *Sanderia*—of which one, *Chrysaora*, was recently revised by Morandini and Marques (2010). Pelagiid jellyfish bearing eight marginal tentacles and eight rhopalia, as the new jellyfish described here, belong either to *Chrysaora* or to the monospecific *Pelagia*. These two genera are easily distinguished by a number of morphological characters. Namely, *Pelagia* have conspicuous exumbrellar cnidocyst warts, shallow rhopaliar pits, 16 marginal lappets, and radial septa terminating between rhopalia and tentacles. By contrast, *Chrysaora* jellyfish have radial septa terminating proximate to the base of the tentacle and a higher number of marginal lappets (32–48 [but see *C. colorata* below]); additional anatomical features are distinctive characters species within *Chrysaora*, such as the occurrence of quadralinga and a heavy manubrium in *C. colorata* and *C. achlyos* (Gershwin & Collins 2002). So far, *C. colorata* is the only known *Chrysaora* species with 8 marginal tentacles, but it is distinguished by its outer morphology (large size and star-shaped exumbrellar marks) from the three nominal *Pelagia* species, the well-known *P. noctiluca* (Forskål 1775), *P. panopyra* Péron & Lesueur 1809, and *P. flaveola* Eschscholtz 1829 (Gershwin and Collins 2002; Cornelius 2013). A striking difference also resides in life history, in the direct metamorphosis planula-ephyra in *Pelagia*, whereas *Chrysaora* species have a polypoid stage.





**FIGURE 5.** *Pelagia benovici* sp. nov. (A–B) Cnidocyst warts (or cnidocyst clubs) of different sizes scattered over the subumbrellar gonads. Oocytes (oo) of different sizes and maturity are shown. (C) Discharged microbasic eurytele (eu). (D) Discharged holotrichous isorhiza (is) and microbasic eurytele (eu). (E) Discharged holotrichous isorhiza (o-is). (F) Microbasic eurytele (eu) and third type of cnidocyst (birh), provisionally identified as heterotrichous microbasic birhopaloid II type (*sensu* Ostman 2000).

A thorough analysis of the older literature (Maas 1903; Mayer 1910; Krasinska 1914; Menon 1930; Kramp 1961; Russell 1964, 1970) revealed the morphological features therefore clearly indicate that the new species must be ascribed to the genus *Pelagia*, and the molecular analysis confirmed its distinctiveness from *P. noctiluca*, with the complication that phylogenetic analyses of 28S suggest incomplete lineage sorting of this more slowly evolving nuclear marker, amplification of paralogues, or hybridization between *P. benovici* and *P. noctiluca*. Each of these three processes could be responsible for the conflict between gene and species trees. So far, besides *P. noctiluca*, all other nominal species of this genus are considered doubtful (Kramp 1961; Gul & Morandini 2013; Cornelius 2013). Indeed, no nominal species of *Pelagia* can be referred to the presently described material (Tab. 2), and *Pelagia benovici* sp. nov. is described here as a new species by a combination of morphological features, notably:

- densely distributed and irregularly shaped (rounded to arrow-pointed) exumbrellar cnidocyst warts (Fig. 7);
- milky white (same colour both in female and male specimens) horse-shoe shaped, outwardly convex gonads (Fig. 7), protruding out of the subumbrellar surface;
- club-shaped cnidocyst warts on gonadal ribbons (Fig. 4D);

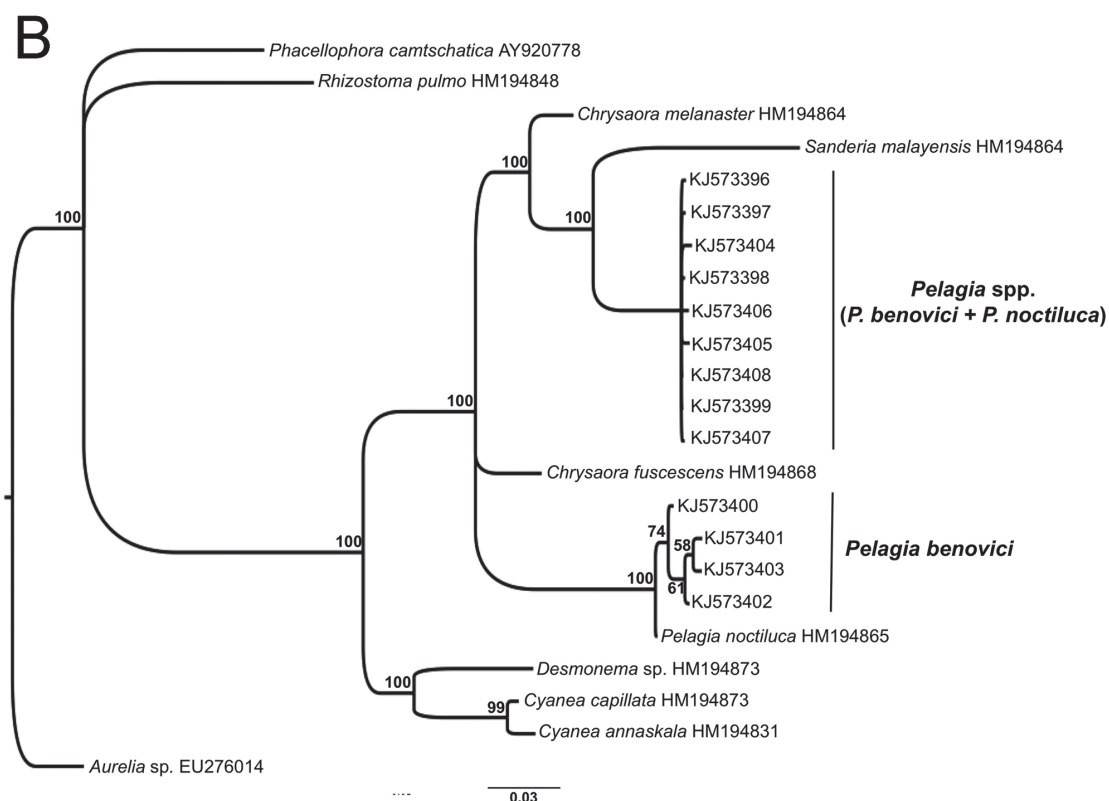


- white transparent colour of tentacles, manubrium and oral arms (Fig. 8), tentacles without longitudinal muscular folds (Fig. 3B).

**TABLE 2.** Comparison of diagnostic characters of species in the genus *Pelagia* (based on Mayer 1910; Kramp 1961; Russell 1964, 1970, Cornelius 1996, 1997; and present work). The asterisk (\*) indicates the minimum size of recorded mature specimens, but the actual size at the onset of sexual maturity still remains unknown.

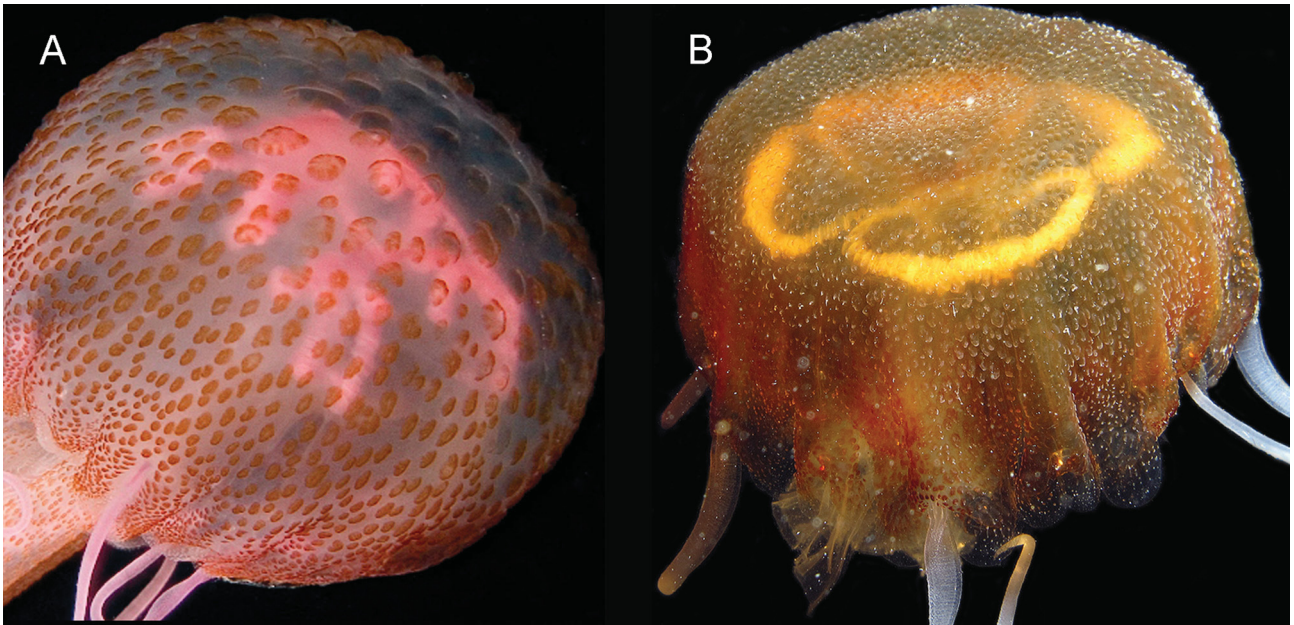
	<i>P. noctiluca</i>	<i>P. flaveola</i> (nomen dubium)	<i>P. panopyra</i> (nomen dubium)	<i>P. benovici</i>
Umbrellar shape and size at maturity (mm)	Hemispherical, thick jelly 35 to 130	Bell rounded, flatter than an hemisphere 16 to 30 (*)	Bell rounded. Width to 50 mm	Flat to hemispherical during active swimming. Thin jelly. 30? to 55 (*)
Color	Exumbrella: mauve to pink to yellowish brown oral arms and tentacles: same as exumbrella. gonads: red to purple	Exumbrella: light brown (jelly colourless and transparent) (oral arms and tentacles) gonads	Colourless to reddish, oral arms speckled rose-red, gonad reddish	Exumbrella: yellow ochre; gonads: milky white
Warts	Rounded and somehow scattered on exumbrellar dome; elliptical, dense on peripheral areas; dome-shaped, uniformly pigmented with cross foldings. Absent on gonad foldings	Colourless, except white tip. Large, thick-set, egg shaped to rounded to somewhat pointed. Height of central dome warts four times the width, becoming progressively less towards the margin	Linear, low surface warts, radially and regularly oriented from exumbrella dome to margin. Less packed than in <i>P. benovici</i>	Rounded to arrow pointed, densely and uniformly packed on exumbrellar dome and periphery. Present also on gonad foldings
Oral arms	Same colour as exumbrella, peripheral areas covered by pigmented warts (same as exumbrella)	Light brown to orange red	Long oral arms, colourless to speckled rose-red	White, transparent. Short oral tube; long delicate oral arms with highly frilled edges. Covered by small transparent cnidocyst warts
Tentacles	Dark-red to pink to brownish, Tentacle proximal base elliptical, including muscular foldings in the mesoglea	Tentacle proximal base elliptical, including muscular foldings in the mesoglea	White to rose Apparently bearing muscular foldings in the mesoglea	White transparent Tentacle proximal base elliptical, without muscular foldings in the mesoglea
Gonads	Purple (male) to reddish (female), convolute foldings in gastric pouches reaching base of manubrium. Outgrowth only on central sectors of gastric pouches.	Light brown, yellowish	Dark-orange to reddish, convolute foldings	Milky white in colour, horse-shoe shaped with peripheral convexity, developed directly on the subumbrellar surface

The findings of sexually mature specimens at sites several hundreds of kilometers apart indicate the possibility of an established population spreading in the North Adriatic. Gershwin & Collins (2002) remarked that pelagiid systematics was oddly neglected, in spite of the conspicuous sizes, distinctive morphologies, and painful stings of these jellyfish. The same authors predicted that new pelagiids likely would be discovered, and *P. benovici* seems a point in case.

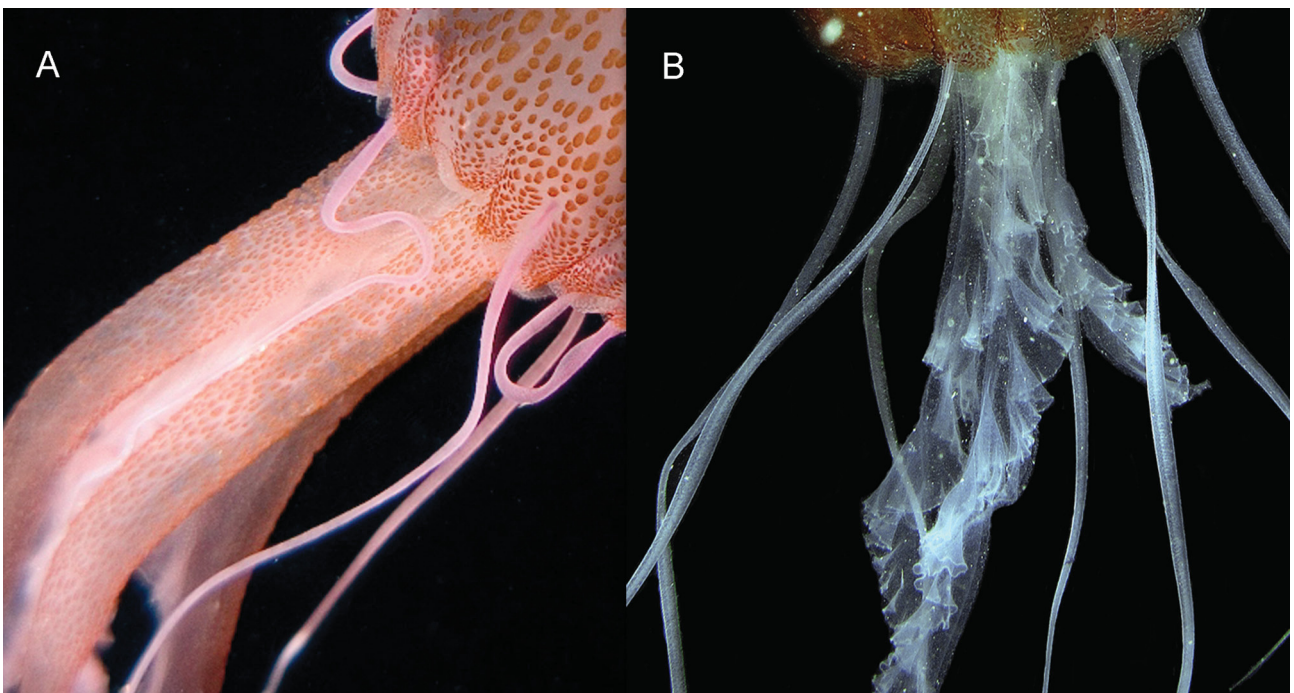


**FIGURE 6.** (A) Bayesian Markov chain Monte Carlo COI gene tree. *Pelagia benovici* sp. nov. sequences group together as a single distinct clade. (B) Bayesian Markov chain Monte Carlo tree (28S sequences). *Pelagia benovici* sp. nov. sequences appear in each of two clades with *P. noctiluca* sequences, suggesting incomplete lineage sorting of this more slowly evolving nuclear marker, amplification of paralogues, or interspecific hybridization. The tree supports the monophyly of Pelagiidae. In both panels, numbers at nodes indicate posterior probabilities, and the scale indicates the number of substitutions per site.





**FIGURE 7.** Appearance of exumbrellar warts, gonad morphology and colour in (A) *Pelagia noctiluca* (A) and (B) *Pelagia benovici* sp. nov.



**FIGURE 8.** Appearance (structure, colour) of oral arms and tentacles in (A) *Pelagia noctiluca* and (B) *Pelagia benovici* sp. nov.

**Native or introduced?** The first alien jellyfish in the Mediterranean Sea, *Cassiopea andromeda*, was found near Cyprus at the beginning of the 20th century (Maas 1903). Since then, three non-native jellyfish species have been recorded in the Mediterranean Sea: *Phyllorhiza punctata* and *Rhopilema nomadica* (Galil 1990), and *Marivagia stellata* Galil and Gershwin 2010 (Galil *et al.* 2010). *R. nomadica* and *M. stellata* also were new to science.

When *M. stellata* was described as a new species from the Israeli Mediterranean coast, Galil *et al.* (2010) cautiously considered it as a non-native species and a probable Lessepsian immigrant, assuming that a native, coastal conspicuous jellyfish, markedly different from all known scyphozoans of the Mediterranean Sea, would

hardly have escaped attention until the 21<sup>st</sup> century. Recently, the finding of *M. stellata* off the coast of Kerala, India (Galil *et al.* 2013) corroborated this hypothesis and established this species as the fourth Erythrean alien jellyfish species introduced in the Mediterranean Sea through the Suez Canal. Similarly, given the number of marine biological stations in the Adriatic Sea, the long history of investigations on gelatinous zooplankton in the area, and the increasing attention on jellyfish blooms in recent years, it is highly unlikely that *P. benovici* remained unnoticed until 2013, when a large population of mature jellyfish suddenly appeared and then persisted in a restricted area. The North Adriatic Sea, particularly the Gulf of Venice, is a major hotspot for introduction of alien species by shipping- and aquaculture-mediated in Europe (Occhipinti *et al.* 2011; Galil 2012) and an increase in shipping related invasions has been noted recently (Galil 2009). Re-discovery of rare jellyfish (e.g. *Rhizostoma luteum* in the Gibraltar Strait or the native *Drymonema dalmatinum* in the Adriatic Sea) after almost a century-long absence (Bayha & Dawson 2010 for a review; Prieto *et al.* 2013) suggests some scyphozoans might remain undetected for a very long time and re-appear suddenly, perhaps linked to the presence of an asexually reproducing polyp stage in the life cycle (Boero *et al.* 2008). However, this is unlikely to be the case for *P. benovici*. The genus *Pelagia* is thought to lack a polyp stage, although this needs to be confirmed for *P. benovici*. By its currently restricted distribution in the Gulf of Venice (Fig. 1), its conspicuous bloom, and each medusa's unignorable size, *P. benovici* seems most likely to be another alien species introduced by human activities into the Mediterranean Sea.

Most probably, *P. benovici* was transported as viable jellyfish in the ballast waters of ships coming from the native area of this species, where it remains still undetected. This is the third case of a new species discovered in the Mediterranean but native to different seas, after the Erythrean immigrants *Alpheus migrans* (Decapoda) Lewinson and Holthuis 1978, and the rhizostome jellyfish *Marivagia stellata*. The life cycle of *P. benovici* still remains unknown, but this new taxon looks like an invasive species with the potential to form large blooms. It may have potential to spread across the Adriatic and neighbouring seas, raising the need for research efforts on mechanisms driving bioinvasions and on the impact of outbreak-forming species, as Boero (2013) recently advocated.

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## References

- Bayha, K.M. & Dawson, M.N. (2010) New family of allomorphic jellyfishes, Drymonematidae (Scyphozoa, Discomedusae), emphasizes evolution in the functional morphology and trophic ecology of gelatinous zooplankton. *Biological Bulletin Woods Hole*, 219, 249–67.
- Bayha, K.M., Dawson, M.N., Collins, A.G., Barbeitos, M.S. & Haddock, S.H. (2010) Evolutionary relationships among scyphozoan jellyfish families based on complete taxon sampling and phylogenetic analyses of 18S and 28S ribosomal DNA. *Integrative and comparative biology*, 50 (3), 436–455.  
<http://dx.doi.org/10.1093/icb/icq074>



- Boero, F. (2013) *Review of jellyfish blooms in the Mediterranean and Black Sea. Studies and Reviews*. General Fisheries Commission for the Mediterranean 92, FAO, Rome, 54 pp.
- Boero, F., Bouillon, J., Gravili, C., Miglietta, M.P., Parsons, T. & Piraino, S. (2008) Gelatinous plankton: irregularities rule the world (sometimes). *Marine Ecology Progress Series*, 356, 299–310.  
<http://dx.doi.org/10.3354/meps07368>
- Brotz, L. & Pauly, D. (2012) Jellyfish populations in the Mediterranean Sea. *Acta Adriatica*, 53, 211–230.
- Canepa, A., Fuentes, V., Sabatés, A., Piraino, S., Boero, F. & Gili, J.M. (2014) *Pelagia noctiluca* in the Mediterranean Sea. In: Pitt, K.A. & Lucas, C.H. (Eds.), *Jellyfish Bloom*. Springer, Netherlands, pp. 237–266.  
[http://dx.doi.org/10.1007/978-94-007-7015-7\\_11](http://dx.doi.org/10.1007/978-94-007-7015-7_11)
- CIESM (2001) *Gelatinous zooplankton outbreaks: theory and practice*. CIESM Workshop series 14, Mediterranean Science Commission, Monaco, 112 pp.
- Cornelius, P.F.S. (1996) *Pelagia flaveola* Eschscholtz 1829 (little brown stinger or little yellow stinger). In: Williamson, J.A., Fenner, P.J., Burnett, J.W. & Rifkin, J.R. (Eds.), *Venomous and Poisonous Marine Animals: A Medical and Biological Handbook*. University of New South Wales Press, Sydney, pp. 230–231.
- Cornelius, P.F.S. (1997) Class Scyphozoa – jellyfish. In: Richmond, M.D. (Ed.), *A guide to the seashores of eastern Africa and the western Indian Ocean islands*. SIDA/Department for Research Cooperation, SAREC, Stockholm, pp. 122–125.
- Cornelius, P.F.S. (2013) *Pelagia* Péron & Lesueur, 1810. Available from: <http://www.marinespecies.org/aphia.php?p=taxdetails&id=135262> (accessed 4 March 2014)
- Dawson, M.N. (2005) *Cyanea capillata* is not a cosmopolitan jellyfish: morphological and molecular evidence for *C. annaskala* and *C. rosea* (Scyphozoa : Semaestomeae : Cyaneidae) in south-eastern Australia. *Invertebrate Systematics*, 19, 361–370.  
<http://dx.doi.org/10.1071/is03035>
- Dawson, M.N. & Jacobs, D. (2001) Molecular evidence for cryptic species of *Aurelia aurita* (Cnidaria, Scyphozoa). *Biological Bulletin Woods Hole*, 200, 92–96.  
<http://dx.doi.org/10.2307/1543089>
- Dawson, M.N., Raskoff, K. & Jacobs, D. (1998) Preservation of marine invertebrate tissues for DNA analyses. *Molecular Marine Biology and Biotechnology*, 7, 145–152.
- Folmer, O., Black, M., Hoeh, W., Lutz, R. & Vrijenhoek, R. (1994) DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology*, 3, 294–299.
- Galil, B.S. (2009) Taking stock: inventory of alien species in the Mediterranean Sea. *Biological Invasions*, 11, 359–372.  
<http://dx.doi.org/10.1007/s10530-008-9253-y>
- Galil, B.S. (2012) Truth and consequences: the bioinvasion of the Mediterranean sea. *Integrative Zoology*, 7, 299–311.  
<http://dx.doi.org/10.1111/j.1749-4877.2012.00307.x>
- Galil, B.S., Kumar, B.A. & Riyas, A.J. (2013) *Marivagia stellata* Galil and Gershwin, 2010 (Scyphozoa: Rhizostomeae: Cepheidae), found off the coast of Kerala, India. *BioInvasions Records*, 2, 317–318.  
<http://dx.doi.org/10.3391/bir.2013.2.4.09>
- Galil, B.S., Spanier, E. & Ferguson, W. (1990) The Scyphomedusae of the Mediterranean coast of Israel, including two Lessepsian migrants new to the Mediterranean. *Zoologisches Mededlingen*, 64, 95–105.
- Galil, B.S., Gershwin, L.-A., Douek, J. & Rinkevich, B. (2010) *Marivagia stellata* gen. et sp. nov. (Scyphozoa: Rhizostomeae: Cepheidae), another alien jellyfish from the Mediterranean coast of Israel. *Aquatic Invasions*, 5, 331–340.  
<http://dx.doi.org/10.3391/ai.2010.5.4.01>
- Gershwin, L.-A. & Collins, A.G. (2002) A preliminary phylogeny of Pelagiidae (Cnidaria, Scyphozoa), with new observations of *Chrysaora colorata* comb. nov. *Journal of Natural History*, 36, 127–148.  
<http://dx.doi.org/10.1080/00222930010003819>
- Gul, S. & Morandini, A.C. (2013) New records of scyphomedusae from Pakistan coast: *Catostylus perezi* and *Pelagia* cf. *noctiluca* (Cnidaria: Scyphozoa). *Marine Biodiversity Records*, 6, e86.  
<http://dx.doi.org/10.1017/s1755267213000602>
- Huang, X. & Madan, A. (1999) CAP3: A DNA Sequence Assembly Program. *Genome Research*, 9, 868–877.  
<http://dx.doi.org/10.1101/gr.9.9.868>
- Kramp, P. (1961) Synopsis of the medusae of the world. *Journal of the Marine Biological Association UK*, 40, 1–469.  
<http://dx.doi.org/10.1017/s0025315400007347>
- Krasinska, S. (1914) Beiträge zur Histologie der Medusen. *Zeitschrift für wissenschaftliche Zoologie*, 109, 256–348.
- Lewinsohn, C. & Holthuis, L.B. (1978) On a new species of *Alpheus* (Crustacea Decapoda, Natantia) from the Eastern Mediterranean. *Zoologisches Mededlingen*, 53, 75–82.
- Maas, O. (1903) Die Scyphomedusen der Siboga Expedition. *Siboga Expedition Monographs*, 11, 1–91.  
<http://dx.doi.org/10.5962/bhl.title.11300>
- Maddison, D.R. & Maddison, W.P. (2005) MacClade 4: Analysis of phylogeny and character evolution. Version 4.08a. Available from: <http://macclade.org> (accessed 25 April 2014).
- Mariscal, R. (1974) Nematocysts. In: Muscatine, L. & Lenhoff, H. (Eds.), *Coelenterate Biology. Reviews and new Perspectives*. Academic Press, New York, pp. 129–178.

- Menon, M.G.K. (1930) The Scyphomedusae of Madras and the neighbouring coast. *Bulletin of the Madras Government Museum*, 3, 1–28.
- Mianzan, H.W. & Cornelius, P. (1999) Cubomedusae and Scyphomedusae. In: Boltovskoy, D. (Ed.), *South Atlantic Zooplankton*. Blackuys Publishers, Leiden, pp. 513–559.
- Miller, B.J., Von der Heyden, S. & Gibbons, M.J. (2012) Significant population genetic structuring of the holoplanktic scyphozoan *Pelagia noctiluca* in the Atlantic Ocean. *African Journal of Marine Science*, 34 (3), 425–430.
- Morandini, A.C. & Marques, A.C. (2010) Revision of the genus *Chrysaora* Péron & Lesueur, 1810 (Cnidaria: Scyphozoa), *Zootaxa*, 97, 1–97.
- Occhipinti-Ambrogi, A., Marchini, A., Cantone, G., Castelli, A., Chimenz, C., Cormaci, M., Frogli, C., Furnari, G., Gambi, M.C., Giaccone, G., Giangrande, A., Gravili, C., Mastrototaro, F., Mazziotti, C., Orsi-Relini, L. & Piraino, S. (2011) Alien species along the Italian coasts: an overview. *Biological Invasions*, 13, 215–37.  
<http://dx.doi.org/10.1007/s10530-010-9803-y>
- Ortman, B.D., Bucklin, A., Pagès, F. & Youngbluth, M. (2010) DNA Barcoding the Medusozoa using mtCOI. *Deep Sea Research Part II Topical Studies in Oceanography*, 57, 2148–2156.  
<http://dx.doi.org/10.1016/j.dsr2.2010.09.017>
- Östman, C. (2000) A guideline to nematocyst nomenclature and classification, and some notes on the systematic value of nematocysts. *Scientia Marina*, 64, 31–46.  
<http://dx.doi.org/10.3989/scimar.2000.64s131>
- Östman, C. & Hydman, J. (1997) Nematocyst analysis of *Cyanea capillata* and *Cyanea lamareckii* (Scyphozoa, Cnidaria). *Scientia Marina*, 61, 313–344.
- Prieto, L., Armani, A. & Macias, D. (2013) Recent stranding of the giant jellyfish *Rhizostoma luteum* Quoy and Gaimard, 1827 (Cnidaria: Scyphozoa: Rhizostomeae) on the Atlantic and Mediterranean coasts. *Marine Biology*, 160, 3241–3247.
- Russell, F.S. (1964) On scyphomedusae of the genus *Pelagia*. *Journal of the Marine Biological Association UK*, 44, 133–136.  
<http://dx.doi.org/10.1017/s0025315400024693>
- Russell, F.S. (1970) *The medusae of the British Isles II. Pelagic Scyphozoa with a supplement to the first volume on Hydromedusae*. Cambridge University Press, Cambridge, 284 pp. Available from: [http://www.mba.ac.uk/nmb/publications/medusae\\_2/medusae\\_2\\_complete.pdf](http://www.mba.ac.uk/nmb/publications/medusae_2/medusae_2_complete.pdf) (accessed 25 April 2014).
- Stopar, K., Ramšak, A., Trontelj, P. & Malej, A. (2010) Lack of genetic structure in the jellyfish *Pelagia noctiluca* (Cnidaria: Scyphozoa: Semaestomeae) across European seas. *Molecular Phylogenetics and Evolution*, 57, 417–428.
- Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M. & Kumar, S. (2011) MEGA5: Molecular Evolutionary Genetics Analysis using Maximum Likelihood, Evolutionary Distance, and Maximum Parsimony Methods. *Molecular Biology and Evolution*, 28, 2731–2739.  
<http://dx.doi.org/10.1093/molbev/msr121>
- Thompson, J.D., Gibson, T.J., Plewniak, F., Jeanmougin, F. & Higgins, D.G. (1997) The CLUSTAL\_X Windows Interface: Flexible Strategies for Multiple Sequence Alignment Aided by Quality Analysis Tools. *Nucleic Acids Research*, 25, 4876–4882.  
<http://dx.doi.org/10.1093/nar/25.24.4876>
- Weill, R. (1934) Contribution à l'étude des Cnidaires et de leurs nématocystes. II: Valeur taxonomique du cnidome. *Travaux de la Station Zoologique de Wimereux*, 10/11, 348–701.